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Speciation and genetic divergence of three species of charr from ancient Lake El'gygytgyn (Chukotka) and their phylogenetic relationships with other representatives of the genus *Salvelinus*

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The diversity of phenotypically different and often reproductively isolated lacustrine forms of charrs of the genus *Salvelinus* represents a substantial problem for taxonomists and evolutionary biologists. Based on the analysis of variability of ten microsatellite loci and two fragments of mitochondrial DNA (control region and *cyt-b* gene), the evolutionary history of three charr species from Lake El'gygytgyn was reconstructed, and phylogenetic relationships between the main representatives of the genus were revealed. Three species from Lake El'gygytgyn were strongly reproductively isolated. Long-finned charr described previously as *Salvethymus svetovidovi*, an ancient endemic form in the lake, originated 3.5 Mya (95% Bayesian credible intervals: 1.7, 6.1). Placement of this species in the phylogenetic tree of *Salvelinus* was not determined strictly, but it should be located in the basal part of the clade *Salvelinus alpinus – S. malma* species complex. The origin of small-mouth charr *S. elgyticus* and Boganida charr *S. boganidae* in Lake El'gygytgyn was related to allopatric speciation. Their ancestors were represented by two glacial lineages of Taranets charr *S. alpinus taranetzi* from Asia. In Lake El'gygytgyn, these lineages entered into secondary contact postglacially. A revision of the main phylogenetic groups within the *Salvelinus alpinus – S. malma* complex is conducted. The Boganida charrs from Lakes El'gygytgyn and Lama (Taimyr) belong to different phylogenetic groups of Arctic charr and should not be regarded as a single species *S. boganidae*. Using the charrs from Lakes El'gygytgyn and Lama as a case study, we show that a model of sympatric speciation, which seemed more probable based on previous empirical evidence, was rejected by other data. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **116**, 63–85.

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INTRODUCTION

The charrs of the genus *Salvelinus* represent one of the most ecologically and morphologically flexible groups of the family Salmonidae. The largest degree of variation is known for Arctic charr *S. alpinus* (L.) and Dolly Varden *S. malma* (Walbaum), as well as for phylogenetically related species and forms designated with the use of such terms as *Salvelinus alpinus* complex (McPhail, 1961; Savvaitova, 1961; Behnke, 1984), *Salvelinus malma* complex (Behnke, 1984, 1989) and *Salvelinus alpinus – Salvelinus malma* complex (e.g. Phillips, Sajdak & Domanico, 1995; Osinov, 2001).

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Additionally, two or more intralacustrine forms that differ in body size, coloration, ecological and feeding niches, and sites and time of reproduction are observed in a large number of lakes within the circumpolar range of the genus (Behnke, 1980; Savvaitova, 1989; Alekseyev, Pichugin & Samusenok, 2000; Jonsson & Jonsson, 2001; Klemetsen, 2010). Based on genetic investigations, the level of reproductive isolation between various forms of charrs within one lake can be different, including strict isolation (e.g. Viktorovsky, 1978; Hindar, Ryman & Stahl, 1986; Osinov, Pavlov & Maksimov, 1996; Gislason *et al.*, 1999; Wilson *et al.*, 2004; Westgaard, Klemetsen & Knudsen, 2004; Adams, Wilson & Ferguson, 2008; Power *et al.*, 2009; Gordeeva *et al.*, 2010; Arbour, Hardie & Hutchings, 2011). The presence of sympatric lacustrine forms with different levels of reproductive isolation observed over a large part of the range of the species complex has led to ongoing discussion on their origin, phylogeny, and taxonomic status (Behnke, 1980, 1984; Nyman, Hammar & Gydemo, 1981; Savvaitova, 1995; Jonsson & Jonsson, 2001).

According to many authors, in the majority of cases, the presence of two or more forms of charrs in the lake is associated with ecological and trophic differentiation, which in some situations can lead to sympatric speciation (Savvaitova, 1995; Smith & Skulason, 1996; Gislason *et al.*, 1999; Robinson & Schluter, 2000; Alekseyev *et al.*, 2000; Jonsson & Jonsson, 2001; Adams & Huntingford, 2004; Knudsen *et al.*, 2006; Adams *et al.*, 2008; Küttner *et al.*, 2013). Recently, some authors have rejected the possibility of sympatric speciation (e.g. Mayr, 1984), including the speciation in charrs (Viktorovsky, 1978). Other researchers consider that sympatric speciation in charrs is possible (e.g. Bolnick & Fitzpatrick, 2007), and mathematical models describing possible scenarios have been suggested (Parker, Noonburg & Nisbet, 2001; Claessen & Dieckmann, 2002; Claessen *et al.*, 2008). It is difficult to see how empirical evidence matches four general (Gavrilets, 2005; Gavrilets *et al.*, 2007) or other possible conditions for sympatric speciation (e.g. Doebeli & Dieckmann, 2005; Rettelbach *et al.*, 2011) identified by theoretical studies. To partially overcome this problem, Coyne & Orr (2004) proposed four empirical criteria for identifying cases of sympatric speciation. Clear cases of sympatric speciation in fish are not numerous, and even these studies are subjected to possible criticism (as with cichlid fish from a Nicaraguan crater lake; Barluenga *et al.*, 2006; Schliewen *et al.*, 2006). A mathematical model has been developed for the verification of sympatric (or parapatric) speciation in cichlids (Gavrilets *et al.*, 2007).

Most of the lakes with two or more forms of charrs appeared during the last postglacial period. Therefore, different lacustrine forms of charrs from these lakes (if their appearance was connected with sympatric speciation rather than with repeated invasions) originated < 10–15 kya. The charrs evolved in some water bodies because of secondary contact and mass hybridization of initially allopatric diverse forms (hybrid swarm theory of adaptive radiation; Seehausen, 2004), which could also have a postglacial origin. A similar scenario is suggested for several lacustrine populations of whitefishes of the genus *Coregonus* (Hudson, Vonlanthen & Seehausen, 2010) and for cichlids of the genus *Steatocranus* (Schwarzer, Misof & Schliewen, 2012). During the glacial period, glacial ice sheets did not cover some lakes, such as Lake El'gygytgyn (Chukotka) and Lake Lama (Taimyr). To take into account the possibility of several invasions and unpredictable consequences of introgressive hybridization, the time and mechanisms of origin of various forms of charrs from these lakes could differ.

Three charr species are known in Lake El'gygytgyn: Boganida charr *S. boganidae* Berg, small-mouth charr *S. elgyticus* Viktorovsky et Glubokovsky (Viktorovsky *et al.*, 1981) and long-finned charr *Salvethymus svetovidovi* Chereshnev et Skopetz. The last-named species is separated into a new monotypic genus based on unique morphological characters (Chereshnev & Skopets, 1990). Based on the opinions of Viktorovsky *et al.* (1981) and Chereshnev & Skopets (1990), small-mouth and long-finned charrs are endemic to Lake El'gygytgyn, and Boganida charr in Lake El'gygytgyn is identical to the same form from Taimyr. General consensus is that the origin of the three species was allopatric and connected with three invasions of ancestor forms to the lake. In particular, the invasion of the ancestor form for long-finned charr occurred in the Pliocene just after appearance of the lake (Chereshnev & Skopets, 1990), and the ancestor forms for two other species invaded the lake during the Pleistocene (Viktorovsky *et al.*, 1981; Glubokovsky *et al.*, 1993; Chereshnev & Skopets, 1993). There are no doubts about the validity of the three charr species from Lake El'gygytgyn (Chereshnev & Skopets, 1993; Glubokovsky *et al.*, 1993; Chereshnev *et al.*, 2002), but different points of view are proposed for their origin and relationships with other charrs of the genus *Salvelinus* (Behnke, 1989; Glubokovsky *et al.*, 1993; Stearley & Smith, 1993; Alekseyev, 2000; Osinov & Lebedev, 2004).

The goals of this study are as follows: (1) to assess the levels of reproductive isolation and genetic divergence between three charr species from Lake El'gygytgyn based on the analysis of variability of ten microsatellite loci and mtDNA sequences of the control region and cytochrome *b* gene; (2) to estimate their

phylogenetic relationships with other charrs of the genus *Salvelinus* with a revision of the main phylogenetic groups within the *Salvelinus alpinus* – *S. malma* species complex; (3) to discuss the taxonomic status of Boganida charrs from Lakes El'gygytgyn (Chukotka) and Lama (Taimyr); and (4) to discuss the most probable scenarios for the origin of the charrs from these lakes, as well as the problems of sympatric speciation in *Salvelinus* and molecular dating.

MATERIAL AND METHODS

Lake El'gygytgyn and its ichthyofauna

Lake El'gygytgyn is located in the Anadyr Plateau of Chukotka at an altitude of 490 m above sea level. It is the only ancient lake on Earth above the Arctic Circle (67°30′N, 172°05′E). Its origin (3.6 Mya) is attributed to a meteorite impact (Gurov & Gurova, 1983; Gurov, Koeberl & Yamnichenko, 2007) or to an explosion of endogenous nature (gas volcanism) (Bely, 1993). The lake bowl is of almost regular rounded shape 12–14 km in diameter with an area of 117.5 km^2. The coastal bank of the lake is 0.5–1.5 km wide with a depth of 10 m, and transitions into the slope at an angle of 30°, and depth increases rapidly up to 169 m in the centre of the lake (Bely, 1993). Four terraces (at an altitude from −10 to +35 or +40 m from the current water level) are located in the lake basin. Their origin is connected to the fluctuations in water level observed over the last 200 kyr (Glushkova & Smirnov, 2007; Fedorov, Shvamborn & Bolshiyanov, 2008). Approximately 50 tributaries flow into the lake and the Enmyvaam River flows from the lake. This river flows into the Belaya River, a tributary of the Anadyr River. The latter river flows into Anadyr Bay of the Bering Sea. Ice covers the lake for a large part of the year (9–12 months). Based on geomorphological data, the area around Lake El'gygytgyn have never been subjected to glaciation (Glushkova, 1993; Glushkova & Smirnov, 2007), and present ecological conditions of the lake are similar to those that occurred during the Pleistocene (Melles *et al.*, 2012). Some inhabitants of the lake, for example many species of diatoms (Kharitonov, 1993), are of ancient origin.

Lake El'gygytgyn is an ultraoligotrophic water body. Its native ichthyofauna is poor, and it is represented mainly by three charr species (Fig. 1). The species differ substantially in body length and weight. In catches conducted from 1985 to 1986, the representatives of Boganida charr were 260–825 (on average, 510) mm in body length and 155–7000 (1703) g in body weight at the age of 9–23 (16) years. These parameters in sexually mature small-mouth and long-finned charrs were: 156–238 (220) mm, 29–114 (81) g and 13–26 years, and 157–330 (270) mm, 34–400 (219) g and 14–30 years, respectively (Chereshnev & Skopets, 1993). These species are characterized by late sexual maturity (11–15 years with mass maturation by 16–20 years). Boganida and small-mouth charrs spawn in the shallows (at a depth of 2–4 m) of the southern and western shores of the lake. The spawning period in small-mouth charr is very short (from late August to early September, exclusively at night), and in Boganida charr it extends from early August to mid September. The exact location of the spawning grounds of long-finned charr has not been determined, but they are probably distributed over the whole slope and hollow of the lake. Sexually mature individuals of this species are observed in catches from June to August, but continuous spawning over the entire year has not been excluded (Chereshnev & Skopets, 1993). The three species can be easily distinguished based on morphology, including characters connected with ecological and trophic specialization (similar to many fish species from northern postglacial lakes) (Robinson & Parsons, 2002; Reist, Power & Dempson, 2013). Boganida charr is a predator feeding mainly on small-mouth and long-finned charrs. Boganida charr are often distributed in the coastal shelf and move to greater depths (up to 100 m) to feed. The fishes are large, with a large head, large mouth and small eyes. The gill rakers are short and thick, and their number on the first branchial arch is 25–31 (on average, 27.5). Small-mouth and long-finned charrs feed mainly on zooplankton, but their foraging habitats are substantially different (Chereshnev & Skopets, 1993). Small-mouth charrs live along the slope, but long-finned charr is observed at the bottom

Figure 1. The charrs of Lake El'gygytgyn (from top): long-finned charr *Salvethymus svetovidovi*, small-mouth charr *Salvelinus elgyticus* and Boganida charr *S. boganidae*. Photographs by M. B. Skopets.

near the lower part of the slope and in the profundal zone. Both species possess a small body size, small head with a small mouth and large eyes. The gill rakers are thin and elongated, and their numbers are 44–53 (47.1) and 44–63 (54.1), respectively. The latter values are maximal not only for charr, but also for all representatives of the subfamily Salmoninae. Boganida and small-mouth charrs possess an elongated body shape, but a comparatively deep body is usual for long-finned charr with a demersal mode of life. In addition to the charrs, the following fish species characterized by a low abundance live in the lake: grayling *Thymallus arcticus*, slimy sculpin *Cottus cognatus*, and (very rarely) chum salmon *Oncorhynchus keta*, round whitefish *Prosopium cylindraceum* and vostrijak whitefish *Coregonus anaulorum*. Representatives of three latter species migrate to the lake from the Enmyvaam River and the Anadyr River basin (Chereshnev & Skopets, 1993).

Sample collection and DNA extraction

Sample material from Lake El'gygytgyn was collected by I.A.C. from June to August 1994. The fishes were sampled with gill nets (of various cell sizes) and spinning rods and reels. Small fragments of muscular tissue or liver were collected in each of 20–25 individuals of the three species. The fragments were fixed in 95% ethanol (for DNA analysis) and in 2-phenoxyethanol (for the analysis of allozyme variability). Genomic DNA was extracted from the ethanol and 2-phenoxyethanol samples by the standard salt method (Aljanabi & Martinez, 1997). In addition, DNA was extracted from fresh frozen or fixed in ethanol tissues (muscles or fins) of different charr forms of the genus *Salvelinus* from various parts of the range (see Supporting Information, Table S4 and Fig. S1). These samples were collected by us or were presented by our colleagues. Material from charrs from Lake Lama was collected in summer 1991 (Osinov *et al.*, 1996).

Microsatellite genotyping and analysis

A total of 55 specimens of three charr species from Lake El'gygytgyn were genotyped by ten microsatellite loci: Smm-3, Smm-22, Smm-24, Smm-21, Smm-17 (Crane *et al.*, 2005), Ssa197 (O'Reilly *et al.*, 1996), SSOSL456 (Slettan, Olsaker & Oystein, 1997), Sco204, Sco205 and Sco218 (DeHaan & Ardren, 2005). The forward primer was modified on the 5′-end by fluorescent dyes FAM, R6G or TAMRA. Amplification reactions were conducted in a final volume of 15 μL using 2.5 μM $MgCl_2$, 100 μM of each dNTP, 1.5 pM of both primers (3 pM for primers Smm24 and Sco218), 10–50 ng DNA and 1 unit of Taq DNA-polymerase (Sileks). Cycling parameters were: initial DNA denaturation at 94 °C for 1 min; 30 cycles of 90 °C – 20 s, 58 °C (in the first cycle, decreasing by 0.2 °C in each cycle) – 25 s, 65 °C – 40 s; seven cycles of 90 °C – 20 s, 52 °C – 25 s, 65 °C – 40 s; and one cycle of 94 °C – 30 s, 45 °C – 90 s, 65 °C for 10 min. Fluorescently labelled fragments were analysed using an ABI 3100 Genetic Analyzer.

Genetic variation within each species was assessed by the indices of allelic richness, A and A_r (corrected for minimum sample size, $N = 8$), the index of private allelic richness A_{pr} (Kalinowski, 2004), observed (H_o) and expected (H_e) heterozygosity using FSTAT 2.9.3.2 (Goudet, 2001), and HP-rare 1.0 (Kalinowski, 2005). The presence of null alleles was checked using the null allele option of GENEPOP 007 software (Rousset, 2008). The calculation of F-statistics (Weir & Cockerham, 1984), Hardy–Weinberg genotypic equilibrium (HWE) for each locus and each sample, as well as the test of linkage disequilibrium among all pairs of loci, was conducted with GENEPOP 007. The level of statistical significance of multiple tests was corrected using the sequential Bonferroni procedure (Rice, 1989). Unbiased estimates of standard genetic distances (Nei, 1987) were calculated in SPAGeDi 1.2 (Hardy & Vekemans, 2002).

The allele size permutation test (1000 randomizations) implemented in SPAGeDi was used to determine whether stepwise mutations contributed to genetic divergence between each species pair (i.e. whether $R_{st} > F_{st}$; where permuted, ρR_{st} was used instead of observed F_{st} values). Although this test was robust for some deviations from the stepwise-like mutation model (SMM) (Hardy *et al.*, 2003), we used two calculation variants for the comparison of *Sl. svetovidovi* vs. *S. boganidae* (or *S. elgyticus*). In the first variant, ten loci were analysed including three Sco loci; and in the second variant, the latter loci were excluded because in two species pairs (*Sl. svetovidovi*/*S. boganidae* and *Sl. svetovidovi*/*S. elgyticus*), the mutation process in Sco loci was distinctly different from that in SMM. In particular, all alleles in *Sl. svetovidovi* were even-sized, whereas the alleles were odd-sized in the two other species (or vice versa), probably caused by fixation of single point indels in the flanking sequence of microsatellites in *Sl. svetovidovi* or in the common ancestor for the two other species.

To detect genetic bottlenecks, the tests from BOTTLENECK 1.2.02 (Piry, Luikart & Cornuet, 1999) were used. Three mutation models were tested: the SMM, infinite allele model (IAM) and two-phase model (TPM). The TPM model comprised 95% single-step and 5% multiple-step mutations, and a variance among multiple steps = 12. The assessments were conducted using 5000 repeats. In addition, the index $M = k/r$ was calculated, where k is the number of

alleles in the locus and $r = S_{max} - S_{min} + 1$ (the difference between the sizes of the largest and smallest alleles expressed in the number of repeats plus 1 to prevent dividing by zero) (Garza & Williamson, 2001; Excoffier, Lava & Schneider, 2005). If the multilocus assessment of this index was lower than a threshold value of 0.68, it indicated that the population passed through a bottleneck (Garza & Williamson, 2001). Based on the calculation procedure suggested by the cited authors, monomorphic loci with $M = 1$ were not included in the analysis. Therefore, the values of the multilocus index M for different populations may be calculated with the use of different numbers and sets of loci. For the calculation of the index based on the standard sample of loci (including monomorphic with the values of the index equal to zero) for all populations, a small modification of the parameter M was applied: $M' = (k - 1)/(S_{max} - S_{min} + 1)$ (Osinov & Gordeeva, 2008). A threshold value of the modified parameter M' was not determined precisely, and it was probably close to 0.49. In addition, the significance of differences for the assessments of A, A_r, A_{pr} and H_e for each species pair of the charrs from Lake El'gygytgyn was determined with the Wilcoxon signed-ranks test.

A Bayesian clustering method implemented in STRUCTURE version 2.3.4 (Pritchard, Stephens & Donnelly, 2000) was used to estimate the number of genetic clusters (K) and membership coefficients (Q) for each of the 55 individuals from Lake El'gygytgyn. The admixture and correlated allele frequencies were used as the ancestry and allele frequency models, respectively. Markov chain Monte Carlo (MCMC) simulations were run for 500 000 generations after 250 000 generations of burn-in and replicated ten times for each value of K from 1 to 4. The value of K corresponding to the highest probability of the data was chosen as the best estimate for the number of genetic clusters. Posterior probabilities of K were computed following the Bayes' rule (Pritchard, Wen & Falush, 2010). In addition, the Delta K method (Evanno, Regnaut & Goudet, 2005) for detecting the number of K that best fit to the data implemented in STRUCTURE HARVESTER software (Earl & von Holdt, 2012) was used.

Mitochondrial DNA (mtDNA) sequencing and analysis

Two fragments of mtDNA [control region (CR) and cytochrome *b* gene (*cyt-b*)] were sequenced in the three charr species fron Lake El'gygytgyn. PCR amplification was performed in 15-μL reaction volumes containing 1× PCR buffer (Sileks), 2.5 mM $MgCl_2$, 600 μM each dNTP, 2 pM each amplification primer, 100 ng DNA and 1 U HotTaq DNA polymerase (Sileks). The primers used for amplification were as follows: for CR, HN20 GTGTTATGCTTTAGTTA AGC and Tpro2 ACCCTTAACTCCCAAAGC (Brunner *et al.*, 2001); and for *cyt-b*, L14795 TAAT GGCCAACCTCCGAAAA and H15844 AGCTACT AGGGCAGGCTCATT (Radchenko, 2005). The fragment of *CR* was sequenced using primer Tpro2, and the fragment of *cyt-b* was sequenced with primers L14795 and H15844. Sequencing was conducted with an automatic ABI 3100 Genetic Analyzer using a BigDye v.1.1 sequence kit. DNA sequences were aligned with CLUSTAL X (Thompson *et al.*, 1997).

Descriptive statistics [haplotype diversity (H_d), nucleotide diversity (π), average number of nucleotide substitutions per site between populations (d_{xy}), number of net nucleotide substitutions per site between populations (d_A) (Nei, 1987) and others] were calculated using DNASP v.5 (Librado & Rozas, 2009). The equation $d_A = 2\lambda t$ (where λ is the rate of nucleotide substitution per site per year) was used for the divergence time estimation. F_{st} estimates were calculated using ARLEQUIN v.3.5 (Excoffier *et al.*, 2005) with pairwise differences.

For the presentation of evolutionary relationships between various haplotypes of a 550-bp fragment of the control region [revealed in the charr species from Lake El'gygytgyn and in other populations of Arctic charr of the Arctic group (Brunner *et al.*, 2001)], a median-joined haplotype network (MJ) (Bandelt, Forster & Rohl, 1999) with default (= 10) weights for all mutations, including deletions, and with parameter epsilon equal to zero, was calculated in NETWORK 4.6.2 (www.fluxus-engineering.com).

Phylogenetic analyses with the maximum-likelihood (ML), maximum-parsimony (MP) and neighbor-joining (NJ) methods were performed using PAUP v.4.0b10 (Swofford, 2002). We used the optimal substitution model GTR + I + G selected by the Akaike information criterion in Modeltest v.3.7 (Posada & Crandall, 1998). The selected model was used for heuristic search with branch-swapping algorithm (TBR) for the best ML tree. The NJ tree was derived from ML distances obtained using a selected substitution model from Modeltest. MP analysis was performed using heuristic search starting with the stepwise additional option (random additional sequences, ten replicates) and TBR algorithm. Gaps were treated as 'missing'. To test for node stability in NJ, ML and MP trees, a non-parametric bootstrap analysis (Felsenstein, 1985) with 1000, 100 and 1000 pseudo-replicates, respectively, was used. In addition, we used the package PhyML 3.0 (Guidon *et al.*, 2010) with parsimony tree as a starting tree and BEST option with GTR + I + G substitution model to find the optimal topology of ML tree. Topological robustness was evaluated using 1000 non-parametric

bootstrap replications and approximate likelihood-ratio test (aLRT) (Guidon *et al.*, 2010).

Bayesian phylogenetic analyses and molecular dating

Bayesian phylogenetic analysis was performed with BEAST v.1.8 (Drummond & Rambaut, 2007) using GTR + I + G substitution model which was found as the best-fit in Modeltest. To allow for rate variation among branches, an uncorrelated lognormal relaxed-clock model (Drummond *et al.*, 2006) was applied. A Yule speciation process as the tree prior was used. Two secondary calibration points obtained during reconstruction of the main stages of the evolutionary history of Salmoniformes and Esociformes (Osinov & Lebedev, 2004) were used for calculation of divergence times (Table S1). The first point was equal to 5.9 ± 1.4 Mya, and it related to the split of the *S. fontinalis*/*S. alpinus* lineages. The second point related to the time of the divergence of two sister taxa, *Salvelinus* and *Oncorhynchus*. Both calibration points were introduced in the analyses assuming a normal distribution centred at 5.9 and 17.8 Mya with a standard deviation of 1.4 and 2.0 Mya, respectively. We did not use the age of Lake El'gygytgyn (3.6 Mya) as a primary calibration point and a maximum age bound for *Sl. svetovidovi*. A position of this species in the phylogenetic tree has not been determined strictly, and we did not introduce different constraints on topology. To check for the possibility of significant rate changes along the tree and to test the global molecular clock hypothesis, we conducted two runs using the random local clock (RLC) model (Drummond & Suchard, 2010). As priors, we used an underlying coalescent process with a constant population size (CP) in the first run and Birth–Death (BD) speciation model in the second run. A Poisson number of rate changes with an expected value of 0.6931 (see Drummond & Suchard, 2010) was applied. We used Tracer1.6 for calculation of the Bayes factor to test a global clock hypothesis according to the RLC model (Suchard, Weiss & Sinsheimer, 2001; Drummond & Suchard, 2010).

All BEAST runs included 30 million MCMC generations with the first three million MCMC steps discarded as burn-in. The samples were drawn every 1000 (or 500) steps and results were checked for acceptable MCMC mixing and sufficient sampling of priors (effective sample size > 200) using Tracer v.1.6 (Rambaut & Drummond, 2007). Node ages [mean values and 95% highest posterior density (95% HPD) id (idem) 95% Bayesian credible intervals (BCIs)] were obtained from maximum clade credibility trees using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk).

RESULTS

Analysis of microsatellite loci in three species from Lake El'gygytgyn

Genetic variability and bottleneck effect

Of ten microsatellite loci analysed, eight were polymorphic in all three species (Tables 1 and S2). The average multilocus number of alleles varied between species from 3.9 in *S. boganidae* to 6.6 in *S. elgyticus*, and allelic richness ranged between 3.0 and 6.0, respectively. The average number of private alleles ranged from 1.47 in *S. boganidae* to 3.31 in *S. elgyticus*. In six loci, *Sl. svetovidovi* shared some alleles with two other species, and in four loci (Ssa197, Sco204, Sco205 and Sco218), all alleles were private. Mean observed and expected heterozygosities were lowest (0.380 and 0.391) in *S. boganidae* and highest (0.535 and 0.663) in *S. elgyticus*. Null alleles were detected in six loci including four loci in *S. elgyticus* and *Sl. svetovidovi* where their frequencies varied from 0.076 to 0.206. In seven cases, deviations from HWE were significant (Table S2). After correction for multiple tests, only three loci deviated significantly from HWE in *Sl. svetovidovi*. The null allele data were not included in the subsequent analysis. Following the correction for multiple tests, linkage disequilibrium was found in the pairs Smm22–Sco204 ($P < 0.05$ in *Sl. svetovidovi*), Smm22–Sco218 ($P < 0.05$ in *Sl. svetovidovi*) and Sco204–Sco218 ($P < 0.01$ in all three species).

Based on Wilcoxon signed-rank tests, significant differences ($P < 0.05$) in A, A_r, A_{pr} and H_e were revealed between *S. boganidae* and *S. elgyticus* and between *S. boganidae* and *Sl. svetovidovi*. Significant

Table 1. Genetic diversity indices for ten microsatellite markers and two mtDNA fragments

	Microsatellites					mtDNA (*CR*/*cyt-b*)			
Species	N	A/A_r	A_{pr}	H_e	M/M'	N	h	H_d	π
S. elgyticus	11.3	6.6/5.96	3.31	0.663	0.74/0.53	17/14	5/7	0.625/0.857	0.00145/0.00143
S. boganidae	19.1	3.9/3.01	1.05	0.391	0.66/0.37	20/20	1/1	0.000/0.000	0.00000/0.00000
Sl. svetovidovi	19.7	6.0/4.65	2.62	0.542	0.70/0.49	18/19	2/2	0.111/0.351	0.00020/0.00033

differences ($P < 0.05$) between *S. elgyticus* and *Sl. svetovidovi* were revealed only in the number of private alleles. M ratio and modified M' ratio estimates were lower than the thresholds (0.68 and 0.49, respectively) in *S. boganidae* (Table 1) presumed to indicate a genetic bottleneck in the past. Among four tests conducted in BOTTLENECK, only the standardized differences test showed a significant deviation from mutation-drift equilibrium in Boganida char when loci fit SMM ($P < 0.01$) and TPM ($P < 0.05$).

Genetic differentiation and hybridization between species

Genetic differentiation between three species pairs based on multilocus estimates of F_{st} and R_{st} were significant ($P < 0.01$, Table 2). The results of allele size permutation tests revealed that the multilocus estimates of observed R_{st} values were significantly higher than permuted ρR_{st} values ($P < 0.01$) for two species pairs (*Sl. svetovidovi*/*S. boganidae* and *Sl. svetovidovi*/*S. elgyticus*). The results of the tests conducted for ten loci (Table 2) or seven loci (data not shown) were similar. Based on the test for the pair *S. boganidae*/*S. elgyticus*, multilocus estimation of R_{st} was higher than that of ρR_{st} ($P < 0.05$) despite it being placed within the 95% CI for ρR_{st} (Table 3). The results suggested a substantial contribution of step-wise mutation to genetic differentiation and of a long period of divergence between the three species. The results are not as clear for the pair *S. boganidae*/*S. elgyticus* in comparison with the other species pairs. Based on the values of standard genetic distances, genetic divergence between *S. boganidae* and *S. elgyticus* was large ($D_s = 0.467$), but it was more than two times lower than that between these two species and *Sl. svetovidovi* (Table 2).

The results from STRUCTURE using the entire data set (55 individuals from three species) indicated that three genetic clusters was the optimal number because of the maximum posterior probability value (Table 3). The value of ΔK for $K = 4$ was not obtained in the STRUCTURE HARVESTER program. Therefore, the Evanno method was not used for the assessment of the optimal number of genetic clusters. All ten replicates for $K = 3$ showed very similar values of estimated membership coefficients for each individual (Q). For the majority of individuals, the values of these coefficients were 0.98–0.99 with minimum values (0.90–0.95) in two individuals (see Fig. 2). Among 55 fishes included in the analysis, hybrids were not found.

Analysis of mtDNA polymorphism

Overall genetic diversity and genetic differentiation

A 550-bp fragment of the control region and a 1053-bp fragment of the *cyt-b* gene were sequenced in 53–55 individuals from Lake El'gygytgyn and in 6–35 individuals from others localities and species (Tables S3, S4). The number of haplotypes revealed using each fragment for a species ranged from one in *S. boganidae* (*CR* and *cyt-b*) to five (*CR*) and seven (*cyt-b*) in *S. elgyticus*. Each of the three species had unique sets of *CR* and *cyt-b* haplotypes. The largest and the lowest assessments of haplotype and nucleotide diversity in both fragments of mtDNA were obtained in *S. elgyticus* and *S. boganidae*, respectively (Table 1). The values of F_{st} calculated for each species pair of the charrs from Lake El'gygytgyn differed significantly from zero ($P < 0.01$) for each fragment (Table 2). Estimates of the average number of nucleotide substitutions per site and the number of net nucleotide substitutions per site between species pairs indicated that genetic divergence between *S. elgyticus* and *S. boganidae* was substantially lower than that between these two species and *Sl. svetovidovi* (Table 2).

Table 2. Observed F_{st} and R_{st}, permuted ρR_{st} (95% CI), standard genetic distances (D_s), number of nucleotide substitutions per site (d_{xy}) and number of net nucleotide substitutions per site (d_A) calculated for ten microsatellite loci and two mtDNA fragments (*CR*, *cyt-b*)

	Microsatellites					F_{st}		d_{xy}/d_A (%)	
Species pair	F_{st}	R_{st}	ρR_{st} (95% CI)	P ($R_{st} > F_{st}$)	D_s	*CR*	*cyt-b*	*CR*	*cyt-b*
S. elgyticus / *S. boganidae*	0.275	0.472	0.258 (0.050–0.504)	0.047	0.467	0.825	0.833	0.27/0.20	0.36/0.29
S. elgyticus / *Sl. svetovidovi*	0.312	0.355	0.111 (0.012–0.273)	0.002	1.152	0.968	0.972	2.65/2.56	2.81/2.72
S. boganidae / *Sl. svetovidovi*	0.467	0.710	0.231 (0.062–0.452)	0.000	1.491	0.997	0.994	2.74/2.72	2.87/2.85

All F_{st} and R_{st} values are significant at $P < 0.001$.

Table 3. Mean log-likelihood values [ln $P(X|K)$] and its standard deviations (SD), posterior probability values [$P(X|K)$] and ΔK values derived from ten replications for different hypothesized numbers of genetic clusters (K) for charrs from Lake El'gygytgyn

	ln $P(X\|K)$			
K	Mean	SD	$P(X\|K)$	ΔK
1	−1804.89	0.8569	0.000	NA
2	−1447.72	27.1209	0.000	6.3
3	−1261.19	0.7636	1.000	333.6
4	−1330.22	136.8433	0.000	NA

NA, not available.

Network of CR haplotypes observed in the Artcic group (sensu Brunner *et al.*, 2001)

As shown previously, the haplotypes of the control region of *S. elgyticus* and *S. boganidae* fall within the clade of the Arctic group (Brunner *et al.*, 2001) that is suggested by our data. However, the haplotypes ARC1 in *S. boganidae*, ARC2 in *S. elgyticus* and ARC3 in *S. alpinus taranetzi* from the Seutakan River (Brunner *et al.*, 2001) are not found in our samples. We suggest that the appearance of these three haplotypes, as well as some others of the Arctic group (Brunner *et al.*, 2001), are connected with sequencing errors. This can be indirectly supported by the apparent discrepancy between haplotype compositions in the samples from geographically similar charr populations from the Canadian Arctic (Brunner *et al.*, 2001; Alekseyev *et al.*, 2009).

In the MJ network (Fig. 3), two main clades of CR haplotypes could be tentatively separated in the representatives of the Arctic group. The first clade included all haplotypes (with the exception of SEU1) observed only in Chukotka (including *S. elgyticus* and *S. boganidae* from Lake El'gygytgyn). The second clade included all haplotypes revealed in North America and (partly) in Greenland. The difference between the central haplotypes for these two clades, Naiv2 and ARC19 (as well as between ARC19 and SEU1), constituted one nucleotide substitution. All haplotypes of small-mouth and Boganida charrs originated from the NAIV2 haplotype, and four from five haplotypes of small-mouth charrs formed a monophyletic group (with a common deletion). The MJ network calculated for *cyt-b* haplotypes revealed in the charrs from Chukotka (data not shown) had a star-like topology with the *S. a. taranetzi* 1 (= NAIV2) haplotype in the centre. This haplotype was observed in Taranets charr from Lake Naivak and in small-mouth charr from Lake El'gygytgyn at high frequency (Table S4).

Phylogenetic relationships in Salvelinus based on *cyt-b* gene sequence data

Phylogenetic trees (NJ, MP, ML and Bayesian) constructed using 1053-bp sequences of the *cyt-b* gene had a similar topology with the exception of several taxa with unstable placements. Alternative placements were usual for the species located in the basal part of the *Salvelinus* tree. In particular, in NJ (data not shown), ML (Fig. 4B) and Bayesian trees (Fig. 5; only the tree obtained with Yule speciation and uncorrelated lognormal molecular clock models was presented), *S. fontinalis* was located near the root, but the same placement was occupied by *S. leucomaenis* in the MP tree (Fig. 4A). However, support for these nodes was low. A topological variant with the placement of *S. fontinalis* near the root and subsequent joining of *S. leucomaenis* was represented in the ML tree [bootstrap support (BP) = 59% and aLRT = 0.70] and in the Bayesian tree (Fig. 5) with a posterior probability (PP) equal to 0.56. In the bootstrap NJ tree, *S. fontinalis* and *S. leucomaenis* were near the root (BP = 70%). The branching patterns for other basal taxa in different methods of phylogenetic analysis (NJ, MP, ML and Bayesian; all runs with different speciation and relaxed molecular clock models) were also different. More often, *S. levanidovi* and then *S. namaycush* (NJ: BP = 84%; MP: BP = 68%; Bayesian CP + RLC: PP = 0.84; Bayesian BD + RLC: PP = 0.94) or a clade (*S. levanidovi*, *S. namaycush*) (Fig. 5) joined after *S. fontinalis* and *S. leucomaenis*. *Salvethymus svetovidovi* or a clade (*Sl. svetovidovi*, *S. malma krascheninnikovi*) joined after these taxa in the majority of trees. The branching pattern when *Sl. svetovidovi* joined to the tree after *S. m. krascheninnikovi* could

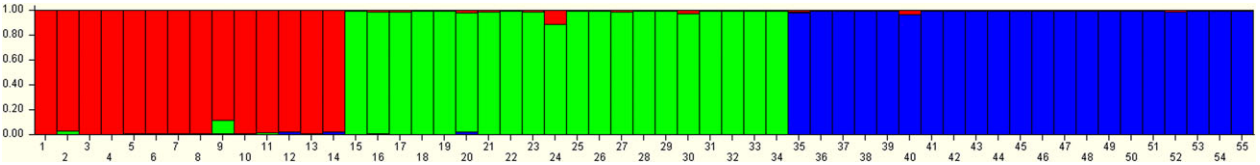

Figure 2. Bar plots of individual membership obtained from STRUCTURE assuming three genetic clusters (K = 3): 1–14, *S. elgyticus*; 15–34, *S. boganidae*; 35–55, *Sl. svetovidovi*.

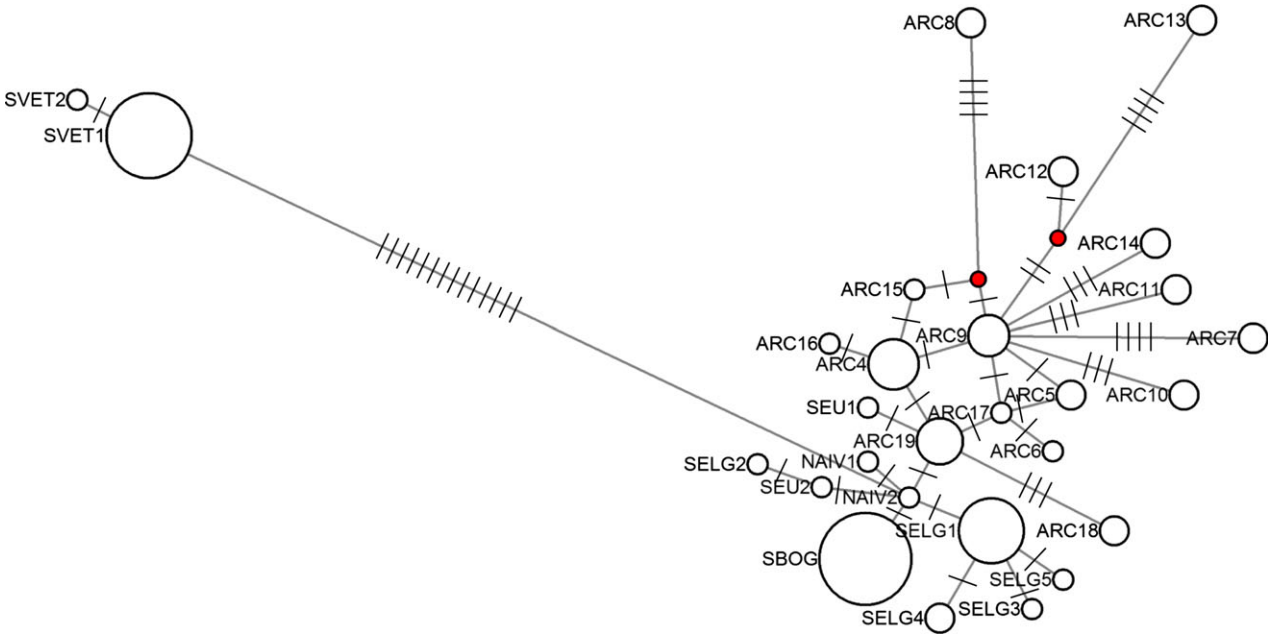

Figure 3. MJ network of the mtDNA control region haplotypes in *Sl. svetovidovi* (SVET), *S. boganidae* (SBOG), *S. elgyticus* (SELG) and *S. alpinus taranetzi* from Chucotka (SEU and NAIV) and North America (ARC). Areas of circles are proportional to the numbers of sampled individuals. Mutations are designated by vertical bars. Haplotype designations as in Table S3.

be found in both the MP tree (BP = 54%) and the Bayesian CP + RLC tree with PP = 0.30.

The largest clade, which included the haplotypes of all taxa of the *S. alpinus – S. malma* complex (without southern Dolly Varden from Asia), had high support values in all phylogenetic trees (see Figs 4, 5). This clade comprised from two clades with two subclades in each. The first clade included the subclade of the haplotypes of Taranets charr *S. a. taranetzi* from North America and Chukotka (Arctic group) and the subclade with the haplotypes of southern Dolly Varden from North America (*S. m. lordi*) and *S. confluentus*. In different trees, the first subclade had moderate or high values of statistical support, and the second subclade was always characterized by lower values (< 50%). The second clade inside the largest clade, as well as two of its subclades, had high support (for the clade: BP = 82–87% and PP > 0.99; for the subclades: BP = 52–77% and PP > 0.93). The first subclade included the haplotypes of northern Dolly Varden *S. m. malma*, and the second subclade included two other subclades. One of the latter subclades was composed of the haplotypes of Arctic charr from Europe (Fennoscandia) (*S. a. alpinus*) and Atlantic coast of North America (*S. a. oquassa*). Another subclade included the haplotypes revealed in the populations of Arctic charr from Taimyr and Transbaikalia (*S. a. erythrinus*). The subclade (*S. a. alpinus*, *S. a. oquassa*) and the subclade of *S. a. erythrinus* haplotypes had high support in all phylogenetic trees (BP = 66–95%, aLRT = 0.75–0.80, PP > 0.95).

Bayesian phylogenetic analyses and molecular dating

As mentioned above, the topologies of different Bayesian trees were mainly similar with the exception of the placement of several basal taxa including long-finned charr. The differences were connected to the replacement of two adjacent branches on the trees that had no pronounced effect on the assessments of molecular dating for certain taxa. The node ages and its 95% BCIs (grey bars) in the tree are illustrated in Fig. 5. Based on analysis with use of the Yule speciation model and uncorrelated lognormal clock model (see Fig. 5), the time of divergence of *Salvelinus* and *Oncorhynchus* reached 15.49 (95% BCIs: 11.7, 19.4) Mya, and the first split in *Salvelinus* was 7.32 (5.2, 9.5) Mya. The divergence time of *S. m. kraschenninnikovi* and *Sl. svetovidovi* was 3.5 (1.7, 6.1) Mya. The mean divergence time for the Arctic group clade was 1.31 (0.46, 2.34) Mya. The mean rate in this Bayesian analysis was 0.0067 (95% BCIs: 0.0044, 0.0092) substitutions per site Myr^{-1}. The divergence times between the three species of Lake El'gygytgyn obtained by these values of substitution rate and d_A distances for the *cyt-b* gene

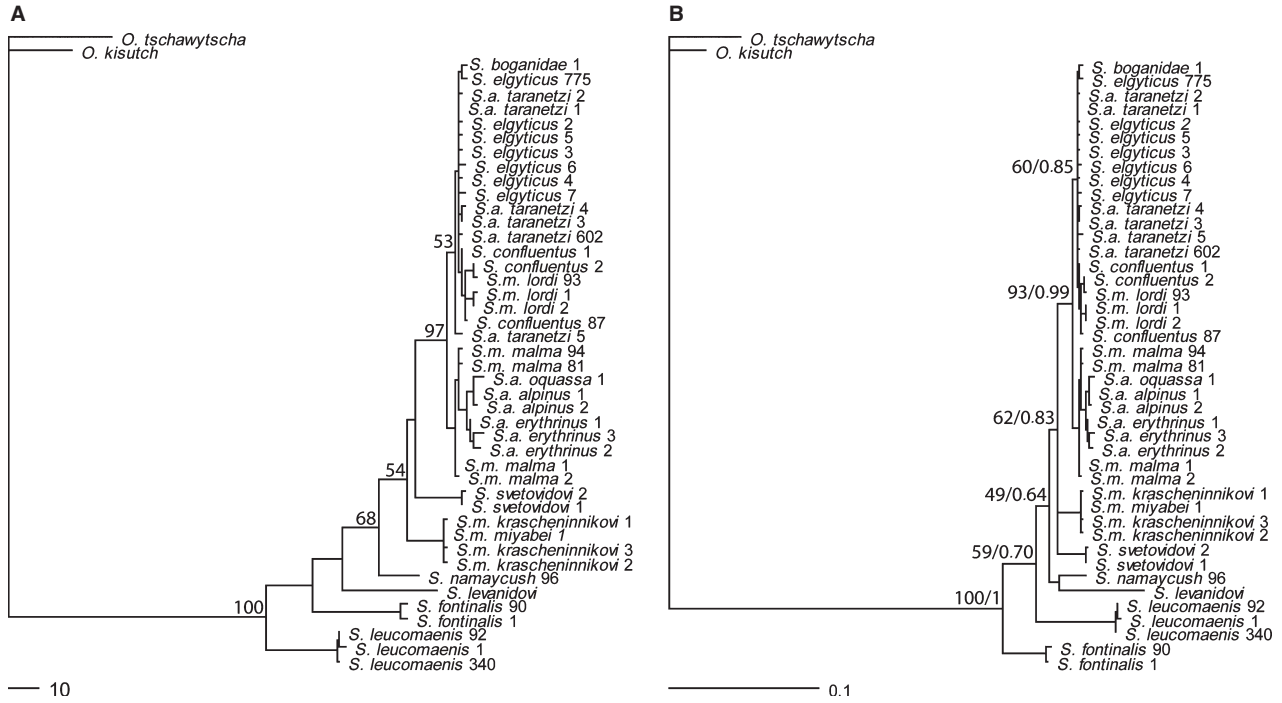

Figure 4. Phylogenetic relationships among *Salvelunus* species inferred from 1053-bp *cyt-b* sequences: A, single MP tree (consistency index = 0.698, retention index = 0.824); B, ML tree based on GTR + G + I substitution model (alpha = 1.597, pinvar = 0.6563). Numbers on branches indicate bootstrap support (BP, in %) in the MP tree and approximate likelihood-ratio test (BP/aLRT) in the ML tree obtained from 1000 pseudoreplicates (BP values < 49% and aLRT < 0.50 are not shown). BP and aLRT indices for some terminal nodes are not shown (see text). Haplotype designations as in Table S4.

(Table 2) were as follows: 2.08 (3.18, 1.55) Mya between long-finned charr and the two other species (on average) and 0.216 (0.329, 0.157) Mya between small-mouth charr and Boganida charr.

The results of the analysis of rate variation among branches of the tree using the RLC model (Drummond & Suchard, 2010) and coalescent process with a constant population size or BD speciation model were as follows. First, 95% BCIs for the branch-specific relative rates for all branches in both trees included a value of 1, suggesting the existence of a global clock. Second, in both runs, a significant part of the posterior mass of the number of rate changes fell on the value = 0 [0.6404 (95% BCIs: 0, 2) for the first run and 0.6060 (95% BCIs: 0, 2) for the second run, respectively]. The Bayes factor estimated using Tracer 1.6 for the rate change count parameter in model comparison was 0.407 and 0.423 for two RLC runs with different speciation models. Based on low values of Bayes factor, the hypothesis of a global molecular clock (Drummond & Suchard, 2010) cannot be rejected. The use of uncorrelated lognormal clock and RLC models showed a small increase in substitution rates for four branches of the tree. These branches were as follows: the branch to *S. levanidovi*, the branch connecting the root with the node of the common ancestor for two *Oncorhynchus* species and the branches from the node to these two species.

DISCUSSION

Genetic differentiation of charrs from Lake El'gygytgyn

Genetic differentiation between three charr species of Lake El'gygytgyn is high, and the microsatellite and mtDNA data suggest their strong reproductive isolation. In our samples, hybrid individuals are not found. Based on the morphological analysis including more than 100 individuals of each species, F1 hybrid individuals also have not been observed (Chereshnev & Skopets, 1993; M. B. Skopets, pers. comm.). The analysis of allozyme variation supports the reproductive isolation between the three species: alternative alleles are fixed in one or two loci (Glubokovsky *et al.*, 1993). Nevertheless, according to allozyme data, the level of genetic divergence among the three species is similar. These data contradict our results from the analyses of microsatellites and mtDNA: the level of genetic divergence between long-finned charr and the two other species is substantially higher than that between the latter species. The discordance between

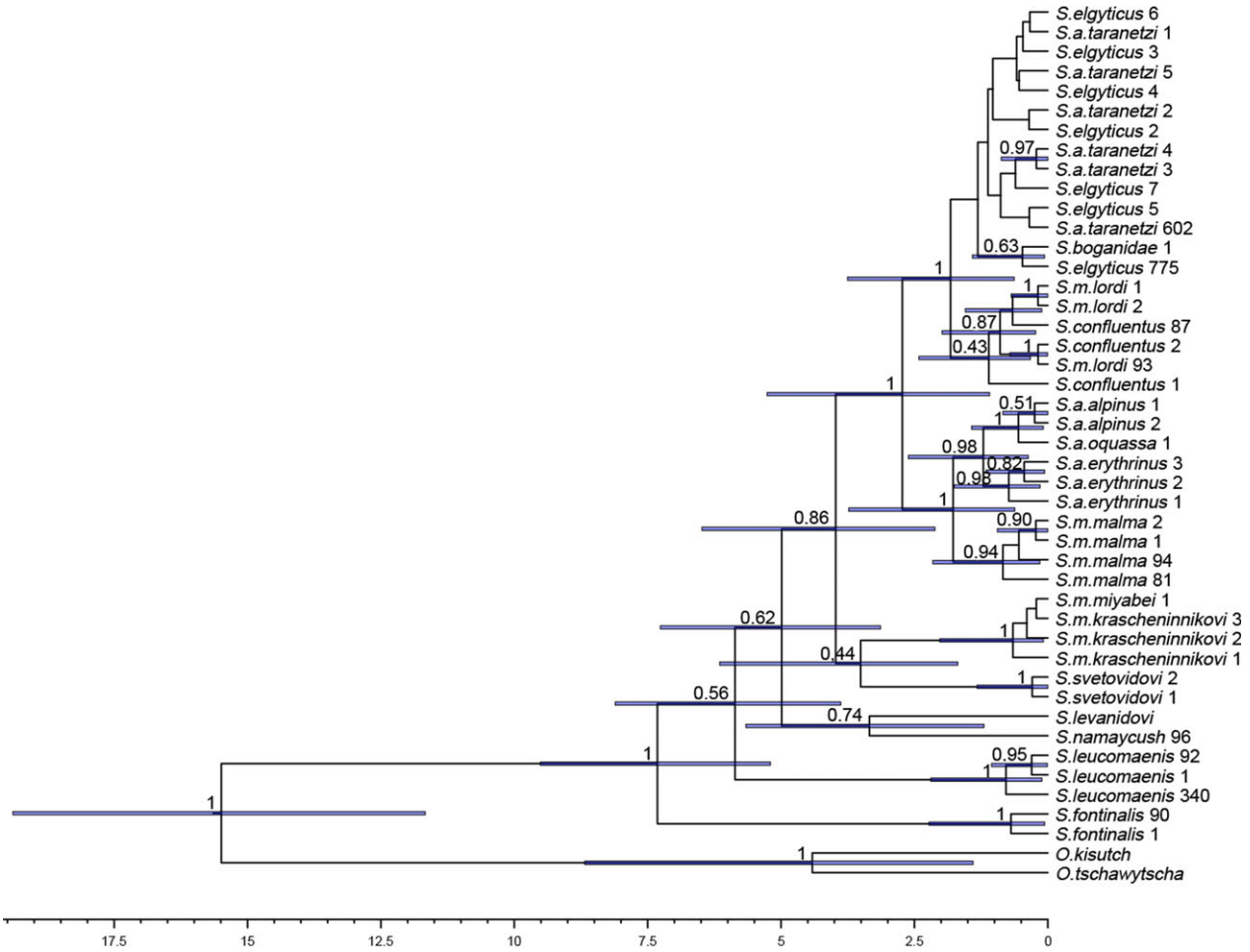

Figure 5. Bayesian maximum clade credibility tree for *Salvelinus* species inferred from 1053-bp *cyt-b* sequences. Numbers near nodes indicate posterior probabilities (those < 0.40 are not shown) and the bars in the nodes represent 95% BCIs for divergence times. Scale bar in Myr from the present time. Haplotype designations as in Table S4.

our and allozyme data (Glubokovsky *et al.*, 1993) can be explained in two main ways. First, it may be connected to possible mistakes during the interpretation of allozyme variation in several loci and to related underestimation of the divergence level between the species (Osinov & Pavlov, 1998). Second, the discrepancy may be associated with past introgressive hybridization between long-finned charr and one or two charr species. Among ten microsatellite loci included in our analysis, 26 alleles (22% from the total allele number in ten loci) are observed in six loci both in long-finned charr and in the two other species. Thus, based on microsatellite data, the possibility of past introgressive hybridization between long-finned charr and one or two charr species from Lake El'gygytgyn cannot be excluded. The data on two fragments of mtDNA do not support hybridization. However, the level of introgression via hybridization can differ significantly among genes (e.g. Martinsen *et al.*, 2001; Chan & Levin, 2005; Petit & Excoffier, 2009; Payseur, 2010). Note that small-mouth charr and Boganida charr possess similar karyotypes [$2n$ = 76–78, chromosome arm number (NF) = 98], which are similar to the karyotypes of Arctic charr from Eurasia and Taranets charr. However, the karyotype of long-finned charr is unique ($2n$ = 56, NF = 98), and it differs from all other *Salvelinus* species in chromosome number (Frolov, 2000).

The low level of genetic variation revealed both in microsatellites and in two mtDNA fragments indicates that Boganida charr has passed through severe bottlenecks. This charr was overfished several times from the beginning of fisheries in Lake El'gygytgyn in the 1950s. Abundance decreased substantially in 1978, and fishing was prohibited from 1986 to 1991, which led to a recovery of the population. However, subsequent overfishing (approximately 10 tonnes or 5000–6000 adult fishes were landed for 5 years) led

to a notable reduction in abundance (Chereshnev *et al.*, 2002). Recovery of numbers has occurred comparatively rapidly. Therefore, a reduction of the effective population size as a result of overfishing, was probably not so significant and prolonged to cause the loss of a large number of alleles of microsatellite loci and mtDNA haplotypes, as well as considerable decrease of average heterozygosity due to genetic drift. We suggest that the decrease of the level of genetic variation in Boganida charr is connected both to the recent drop of its abundance as a result of overfishing and with to of the more distant past.

Phylogenetic relationships between charrs of the genus *Salvelinus* and systematic position of *Salvethymus svetovidovi*

At present, the number of species of the genus *Salvelinus* and their phylogenetic relationships are have not been established reliably. We consider a group of 'good' species and a group of the species and forms with comparatively recent origin and unclear taxonomic status. The latter group phylogenetically related to Arctic charr (*S. alpinus*) and Dolly Varden (*S. malma*) is often regarded as a species complex (Behnke, 1984, 1989; Savvaitova, 1989; Crane, Seeb & Seeb, 1994; Phillips *et al.*, 1999; Brunner *et al.*, 2001; Osinov, 2001). The 'good' species are usually located in the basal part of the *Salvelinus* phylogenetic tree based on morphological, allozyme, karyological and molecular data. These species are: *S. fontinalis*, *S. namaycush*, *S. leucomaenis*, *S. confluentus* and *S. levanidovi* (Cavender & Kimura, 1989; Chereshnev, Skopets & Gudkov, 1989; Stearley & Smith, 1993; Crespi & Fulton, 2004; Osinov & Lebedev, 2004; Oleinik, Skurikhina & Brykov, 2007; Wilson & Williams, 2010; Shedko, Miroshnichenko & Nemkova, 2013). Not all authors agree with the opinion that *Sl. svetovidovi* from Lake El'gygytgyn is the most similar to the ancestral form for the charrs of the genus *Salvelinus* (Chereshnev & Skopets, 1990) and its separation into a distinct genus is reliably justified (Behnke, 1989; Stearley & Smith, 1993; Glubokovsky *et al.*, 1993; Alekseyev, 2000; Osinov & Lebedev, 2004). Based on karyological data, Frolov (2000) placed this charr near the root of the phylogenetic tree of *Salvelinus*. According to Alekseyev (2000), morphological and osteological characters used for the separation of the species into a distinct genus *Salvethymus* (Chereshnev & Skopets, 1990) probably represent a consequence of paedomorphosis, and karyological data (Frolov, 2000) can be interpreted in two ways. In Alekseyev's opinion, an ancient origin and taxonomic status of long-finned charr, as well as a suggested scenario for the evolution of the three species from Lake El'gygytgyn (Chereshnev & Skopets, 1990), should be reconsidered.

To resolve phylogenetic relationships between all main representatives of the genus *Salvelinus*, all charr species mentioned above and representatives of the *S. alpinus* – *S. malma* complex should be analysed. However, such investigations are very rare. For example, only in two studies (Shedko *et al.*, 2013; this study) from a large number of molecular genetic investigations have such analysis been conducted (e.g. Grewe, Billington & Hebert, 1990; Phillips & Oakley, 1997; Brunner *et al.*, 2001; Westrich *et al.*, 2002; Crespi & Fulton, 2004; Radchenko, 2005; Oleinik *et al.*, 2007; Shedko *et al.*, 2007; Taylor *et al.*, 2008; Crete-Lafreniere *et al.*, 2012). Note that a comparative analysis of phylogenetic studies is difficult: different data sets can lead to various topologies of phylogenetic trees for many reasons. In addition, a problem of taxon sampling (Wiens, 2005; Heath, Hedtke & Hillis, 2008) should be taken into account.

Phylogenetic relationships between basal taxa of *Salvelinus* have not yet been resolved. According to our data, *S. fontinalis* (ML, NJ and Bayesian) or *S. leucomaenis* (MP) are located near the root of the *Salvelinus* phylogenetic tree. However, high statistical support is not obtained for either variant. Besides these two variants, a basal placement of the clade (*S. fontinalis*, *S. leucomaenis*) or subsequent joining of *S. levanidovi* and then *S. leucomaenis* after *S. fontinalis* are reported (Crete-Lafreniere, Weir & Bernatchez, 2012; Shedko *et al.*, 2013). Recently, *S. fontinalis* and *S. namaycush* have been recognized as sister species and they are placed near the root of the *Salvelinus* tree based on the analysis of morphological, karyological and molecular data (in the absence of *S. levanidovi* and *Sl. svetovidovi* for the analysis) (see Crespi & Fulton, 2004). In our phylogenetic trees, *S. levanidovi* and then *S. namaycush* or the clade (*S. levanidovi*, *S. namaycush*) join to the tree after *S. fontinalis* and *S. leucomaenis*. Based on other molecular (Radchenko, 2005; Oleinik *et al.*, 2007; Shedko, Miroshnichenko & Nemkova, 2012) and karyological (Frolov, 2000) data, *S. levanidovi* is one of the basal species of the genus *Salvelinus*. A placement of *Sl. svetovidovi* in the phylogenetic tree is not strictly defined. This species probably represents a sister taxon for the southern form of Dolly Varden from Asia (DVSAsia) or for the clade *S. alpinus* – *S. malma* complex (without DVSAsia). This assumption is in agreement with the previous conclusion based on analysis of the mtDNA control region (Brunner *et al.*, 2001; Shedko *et al.*, 2007; Taylor *et al.*, 2008) and other data (Crete-Lafreniere *et al.*, 2012) to take into account the absence of

DVSAsia in two of these studies. Thus, *Sl. svetovidovi* should probably be placed within the *S. alpinus* – *S. malma* complex, and its origin is connected to the onset of divergence inside this complex, but not to the divergence in the whole genus as suggested by Chereshnev & Skopets (1990). Regarding the systematic position of long-finned charr, this taxon should be included in the genus *Salvelinus* (according to phylogenetic systematics, the taxa should be monophyletic) (Osinov & Lebedev, 2004).

Main phylogenetic groups in the *Salvelinus apinus* – *Salvelnus malma* species complex

The phylogeny and taxonomy of the species and forms that have been combined by different authors into the *S. alpinus* complex, *S. malma* complex or *S. alpinus* – *S. malma* complex is a subject for intensive discussions related to fishes of the family Salmonidae (e.g. Behnke, 1980, 1984, 1989; Savvaitova, 1989, 1995; Glubokovsky *et al.*, 1993; Osinov, 2001; Osinov & Lebedev, 2004). In particular, according to several authors (Barsukov, 1960; Savvaitova & Volobuev, 1978), the species status of Dolly Varden is doubtful, and all various forms of Arctic charr and Dolly Varden should be combined into the *S. alpinus* complex (Savvaitova, 1989). Behnke recognized the validity of *S. alpinus* and *S. malma*, but his opinions on the composition of these species and related species complexes, as well on their phylogenetic relationships with some other charr species, have changed (compare Behnke, 1980, 1984, 1989). For example, the northern form of Dolly Varden (*S. malma malma*) from Asia and North America, southern form of Dolly Varden from Asia (*S. m. kraschenninnikovi*) and southern form from North America (*S. m. lordi*) were initially included in the Dolly Varden species complex (Behnke, 1984). The stone char (*S. albus*) from the Kamchatka River, *S. leucomaenis* and *S. confluentus* were also included in the complex. The two latter species were later excluded from the complex (Behnke, 1989).

Based on our data, the most important aspects of the phylogeny of the *S. alpinus* – *S. malma* complex are as follows. Southern Dolly Varden from Asia has a basal position (see also Shedko *et al.*, 2007; Taylor *et al.*, 2008). This conclusion is similar to that obtained previously based on allozyme data: southern Dolly Varden from Asia is the most similar to the ancestral form, and the initial centre of origin of this species complex is referred to Pacific drainages (Osinov & Pavlov, 1998; Osinov, 2001). As suggested previously (Brunner *et al.*, 2001), our data indicate that the clade of northern Dolly Varden halpotypes (*S. m. malma*, Bering group) is a sister group to the clade of Arctic charr haplotypes from Siberia (*S. a. erythrinus*, Siberian group), Europe (*S. a. alpinus*, Atlantic group) and Atlantic coast of North America (*S. a. oquassa*, Acadia group). The haplotypes of southern Dolly Varden from North America (*S. m. lordi*) together with the haplotypes of *S. confluentus* form the clade sister to the clade including the haplotypes of high Arctic charr from Arctic Canada and Chukotka (*S. a. taranetzi*), *S. elgyticus* and *S. boganidae* from Lake El'gygytgyn. Thus, each of the three forms of Dolly Varden (Behnke, 1980, 1984) possesses its own clade of *cyt-b* haplotypes, but these clades are not combined into one large clade, and a monophyletic origin of Dolly Varden lineage, as well as of Arctic charr lineage, is not supported.

Our findings again confirm that phylogenetic relationships between several taxa of charrs revealed by the analyses of mtDNA and allozymes can differ (Osinov, 2002). Based on allozyme data, different forms of Arctic charr on the one hand and three forms of Dolly Varden on the other probably represent two monophyletic lineages (Crane *et al.*, 1994; Osinov & Pavlov, 1998; Salmenkova *et al.*, 2000; Osinov, 2001). A gene tree does not necessarily correspond to a species tree for several reasons, including a case of introgressive hybridization (Funk & Omland, 2003). Introgression of mtDNA is reported for different groups of animals (Toews & Brelsford, 2012) including charrs of the genus *Salvelinus* (Glemet, Blier & Bernatchez, 1998; Wilson & Bernatchez, 1998). Available genetic data suggest several cases of introgressive hybridization between different taxa of *Salvelinus* and related problems connected with these cases. For example, the analysis of sequences of the control region of mtDNA shows that the haplotypes of three forms of Dolly Varden form a single clade (Bering), and an origin of these forms from the population of a glacial refugium is proposed (Brunner *et al.*, 2001). As later shown by Shedko *et al.* (2007), all haplotypes of the Bering group described by Brunner an co-authors belong to northern Dolly Varden, and their appearance (with high frequencies) in the populations of southern Dolly Varden from Asia is associated with introgressive hybridization. This hybridization probably occurred during postglacial expansion of the northern Dolly Varden (Osinov & Mugue, 2008).

A problem related to the origin and phylogenetic placement of southern Dolly Varden from North America has not yet been resolved. Genetic data are contradictory, connected to possible introgressive hybridization in the past (a long time ago and recently) with probable participation of northern Dolly Varden, Taranets charr and bull trout. A substitution of 'own' mtDNA to 'alien' mtDNA in bull trout and southern Dolly Varden from North America cannot be excluded. However, based on available

data, the indication of a donor or recipient seems difficult (e.g. Phillips & Oakley, 1997; Brunner *et al.*, 2001; Osinov, 2001; Redenbach & Taylor, 2002; Elz, 2003; Shedko *et al.*, 2007; Taylor *et al.*, 2008; Crete-Lafreniere *et al.*, 2012).

A hypothesis on a replacement of native mtDNA by mtDNA of Arctic charr (Arctic group) in northern Dolly Varden at an early stage of its evolution has been proposed (Shedko *et al.*, 2007). This assumption seems possible, but the donor of mtDNA is probably Arctic charr from Eurasia. As mentioned above, based on allozyme (Osinov & Pavlov, 1998), ITS1 (Phillips *et al.*, 1999) and RAG1 (Shedko *et al.*, 2012) data, northern Dolly Varden is a sister group to southern Dolly Varden from Asia, and according to mitochondrial data (Brunner *et al.*, 2001; Radchenko, 2005; this study), this form represents a sister group to Arctic charr from Siberia and Europe.

Our data on the variability of sequences of a 1053-bp fragment of the *cyt-b* gene and 550-bp fragment of the control region (see also Brunner *et al.*, 2001), as well as allozyme data (Osinov *et al.*, 1996; Osinov, 2001), suggest a phylogenetic similarity between the populations of Arctic charr from Europe (*S. alpinus alpinus*, Atlantic group), North America (*S. a. oquassa* from Maine and Quebec, Acadia group) and Siberia (*S. a. erythrinus*, Siberia group). It is necessary here to make an important clarification because the ranges of Atlantic and Siberian groups (particulary in Taimyr) appears more complex than previously suggested (Brunner *et al.*, 2001; Osinov, 2002; see the Lake Lama section in Supporting Information, Data S1 for details). Based on the analysis of the control region of mtDNA, the haplotypes of a single phylogenetic group (Siberia) are revealed in the charr populations from Taimyr and Transbaikalia (Brunner *et al.*, 2001). However, the new mtDNA data obtained from different studies (Radchenko, 2003; Alekseyev *et al.*, 2009; this study) clearly indicate a secondary contact between the populations of Atlantic and Siberian phylogenetic groups of Arctic charr in Taimyr.

These results show that the inclusion of the populations from Taimyr (Atlantic and Siberian groups) and Transbaikalia (probably only Siberian group) together with the populations of high Arctic charr from North America (to the east of the Mackenzie River, Arctic group) in *S. a. erythrinus* (Behnke, 1984) is erroneous. Based on our data on the mtDNA control region, Arctic charr from Chukotka (Taranets charr) is related to high Arctic charr from Canada, contradicting the suggestion (Brunner *et al.*, 2001) of the substantial genetic divergence of these forms. Nevertheless, a distribution of Taranets charr from Chukotka and high Arctic charr from North America in different refugia during the last glaciation seems very probable. Small-mouth charr and Boganida charr from Lake El'gygytgyn are phylogenetically similar with Taranets charr from Chukotka. Our data support the suggestions proposed at different times and with varying degrees of confidence about the phylogenetic similarity between Taranets charr from Asia and high Arctic charr populations to the east and lacustrine populations to the west of the Mackenzie River (Behnke, 1980; Glubokovsky & Chereshnev, 1981; Osinov, 2001). However, due to the restricted genetic and molecular data on lacustrine charr populations from Alaska (Reist, Johnson & Carmichael, 1997; Taylor *et al.*, 2008), additional investigations are needed.

Available allozyme data and various molecular data indicate that the *S. alpinus* – *S. malma* species complex (a part of long-finned charr) is composed of two main phylogenetic lineages. The Dolly Varden lineage includes two phylogenetic groups represented by northern and southern (Asian) forms. The Arctic charr lineage is composed of four phylogenetic groups, which (based on the analysis of a 550-bp fragment of the control region) were designated as Atlantic, Acadia, Siberia and Arctic (Brunner *et al.*, 2001). Based on the present data, the southern form of Dolly Varden from North America cannot be realiably referred to a certain lineage. Some of the phylogenetic groups are regarded as subspecies of *S. alpinus* and *S. malma* (Behnke, 1980, 1984) or as different species (other authors).

Speciation in the charrs from Lake El'gygytgyn and the problems of sympatric speciation

High phenotypic plasticity is observed in several charr species of the genus *Salvelinus*, especially in Arctic charr, which has led to serious problems for the scientists who have examined their systematics and evolutionary history (e.g. Behnke, 1980, 1984, 1989; Savvaitova, 1989, 1995; Osinov, 2001). There are many lakes with from two to four sympatric forms of charrs, and some of them are reproductively isolated (e.g. Gislason *et al.*, 1999; Wilson *et al.*, 2004; Adams *et al.*, 2008; Gordeeva *et al.*, 2010; Garduno-Paz *et al.*, 2012). Until recently, among many observations of the presence of several sympatric forms of Arctic charr in a lake, only one (Gislason *et al.*, 1999) has been considered as a certain case of sympatric speciation (see reviews by Coyne & Orr, 2004; Bolnick & Fitzpatrick, 2007).

Lake El'gygytgyn is the ancient lake with three charr species located above the Arctic Circle. Different opinions have been given for their origin and phylogeny (Chereshnev & Skopets, 1990, 1993; Behnke, 1984, 1989; Glubokovsky *et al.*, 1993;

Alekseyev, 2000; Osinov & Lebedev, 2004). Placement in the phylogenetic tree and molecular dating support an ancient origin of *Sl. svetovidovi* in this lake (Chereshnev & Skopets, 1993). The mtDNA sequence data are in conflict with the assumption (Behnke, 1984; Glubokovsky *et al.*, 1993) that Boganida charr and small-mouth charr have different evolutionary ages and represent phylogenetically distant groups. Small-mouth and Boganida charrs from Lake El'gygytgyn are closely related to Taranetz charr from Chukotka. Based on MJ network analysis, the haplotype (*CR* and *cyt-b* gene) revealed in one of two individuals of Taranetz charr from Lake Naivak is regarded as the ancestral haplotype for all haplotypes of small-mouth and Boganida charrs. These results indirectly indicate that a single colonization event of the ancestral lineage of Taranetz charr from Chukotka occurred in Lake El'gygytgyn during the postglacial period.

We suggest that the appearance of two sister charr species in Lake El'gygytgyn is connected to the process of trophic and ecological differentiation as a consequence of ontogenetic niche shift in diet and habitat in a size-structured population (Werner & Gilliam, 1984) according to the model of sympatric speciation (Claessen & Dieckmann, 2002). As a result, two charr species specialized in feeding type have appeared. One of them, small-mouth charr, developed a small body size and adaptive changes (e.g. increased number of elongated gill rakers) associated with the transition to feeding exclusively on zooplankton. Representatives of this species occupied upper pelagic water layers of the lake. Their food competitor, long-finned charr, remained in its former, glacial-age habitats. Another species, Boganida charr, characterized by a large body size and adaptive changes (e.g. well-developed teeth on the jaws), transitioned to obligatory predation. There are many examples of ontogenetic shift of food objects and the patterns of trophic specialization in charrs. These processes led to the origin of different lacustrine forms and to their subsequent ecological differentiation (Amundsen, 1994; Snorrason *et al.*, 1994; Skulason *et al.*, 1996; Smith & Skulason, 1996; Jonsson & Jonsson, 2001; Adams & Huntingford, 2004; Knudsen *et al.*, 2006; Amundsen, Knudsen & Klemetsen, 2008).

Molecular data support that all three species are strongly reproductively isolated, and, at present time, hybridization between them is absent. This means that speciation is complete, and a complex of mechanisms of reproductive isolation including premating and postzygotic is established. Both the spawning time and spawning grounds in Boganida and small-mouth charrs near the Enmyvaam River head partly overlap. However, crossing between these species is not observed, and, if it occurs, no F1 hybrids are viable. It is possible that the body size in these two species is a 'magic trait' that is both subject to divergent selection and controls non-random mating (Gavrilets *et al.*, 2007; Thibert-Plante & Gavrilets, 2013).

Empirical evidence meets all four criteria for the identification of sympatric speciation (Coyne & Orr, 2004) in Boganida charr and small-mouth charr from Lake El'gygytgyn. First, both species are endemic forms for this lake and possess completely overlapping geographical ranges. Second, speciation has been completed. Third, they are sister species and have a monophyletic origin. Fourth, according to mtDNA data, a single colonization event from an ancestral population of Taranets charr from Chucotka occurred in the lake. Therefore, sympatric speciation is the most parsimonious scenario for the origin of small-mouth and Boganida charrs in Lake El'gygytgyn (based on *CR* and *cyt-b* sequence data). However, restriction fragment length polymorphism (RFLP) data of Radchenko (2003) reject the possibility of sympatric speciation of these species. The analysis of a 2162-bp ATPase6/ND4L region of mtDNA using *Mva*I (one of 12 restriction enzymes used in the study) shows that all individuals of small-mouth charr from Lake El'gygytgyn ($N = 13$) and all individuals from Maksi ($N = 3$) and Juliette lakes ($N = 25$) from the Bayunda River basin (upper reaches of the Kolyma River) share the restriction haplotype MvaI-A. All individuals of Boganida charr from Lake El'gygytgyn ($N = 22$) and all Taranets charrs ($N = 21$) from the lakes of the Vykvynaivaam River basin (Chukotka) possess RFLP haplotype MvaI-B. Two important conclusions can be proposed based on these data. First, the group of Taranets charr from Chukotka separated based on CR sequences data includes two glacial lineages from Asia. Second, the origin of small-mouth and Boganida charrs is connected to the invasion of two forms of Taranets charr into Lake El'gygytgyn during the postglacial period. According to the mtDNA data (both RFLP and sequences), introgressive hybridization between these forms after their secondary contact in the lake was minimal.

It could be supposed that Lake El'gygytgyn was a refugium for one of these forms, but this suggestion seems not to be reliable. The spawning grounds of small-mouth and Boganida charrs are in the shallows (depths of 2–4 m) of the southern and western coasts of the lake (Chereshnev & Skopets, 1993). The water level of the lake was 9–10 m lower than the present water level from 10 to 20 kya (Fedorov *et al.*, 2008), and today's shallow part of the lake with spawning grounds of the two species was absent. In addition, we suggest that the distribution of this

form over the range (in Chukotka or Kolyma River basin) began from the Lake El'gygytgyn basin during the postglacial period. The small-mouth charr is a (morphologically and ecologically) specialized species. Therefore, it is more probable that small-mouth charr originated from more generalized populations of Taranets charr invaded Lake El'gygytgyn from the Kolyma River basin than vice versa. As already suggested, Boganida charr entered into Lake El'gygytgyn through water connection with the Kolyma basin (which appeared during the Late Pleistocene) and then migrated to the upper reaches of the Anadyr basin where it remained in lakes Pennoe and Baran'e (Chereshnev & Skopets, 1993). However, the parasites usual for Boganida charr and two other charr species from Lake El'gygytgyn (e.g. cestodes *Eobotrium salvelini*) are absent in the Boganida charr from Lake Pennoe (Atrashkevich & Orlovskaya, 1993). This problem can be solved if we suggest that colonization of Lake El'gygytgyn by the ancestor for Boganida charr has occurred in the opposite direction (i.e. through the Anadyr basin, and the parasites initially were absent). The invasions of Taranets charr and northern Dolly Varden into the Anadyr River basin probably occurred approximately 9–10 kya: the Bering Strait used for the southward distribution of these charrs opened approximately 10 500 years ago (Elias *et al.*, 1996). Thus, the colonization of Lake El'gygytgyn by two glacial lineages of Taranets charr probably occurred both from the Kolyma River basin (ancestor of small-mouth charr) and the Anadyr River basin (ancestor of Boganida charr). Both invasions took place during postglacially, but the time difference between them could reach from several hundred to several thousand years.

Contrary to Viktorovsky (1978), we believe that sympatric speciation in charrs is possible. However, as we show from the examples of Lakes El'gygytgyn (Chukotka) and Lama (Taimyr) (see the Lake Lama section in Supporting Information, Data S1), the empirical data, which seem to support sympatric speciation, are not always reliable. It seems possible that a careful analysis of other reported cases of sympatric speciation in charrs [including the charrs from Lake Galtabol (Gislason *et al.*, 1999)] will show that many such cases should be rejected.

SALVELINUS BOGANIDAE AND A PROBLEM OF TAXONOMY IN *SALVELINUS*

Boganida charr *S. boganidae* Berg from Lake Boganidskoe (the Khatanga River basin) and Dryagin's charr *S. drjagini* Logaschev from Lake Melkoe (the Pyasina River basin) are widely distributed in Taimyr, including lakes Lama and Sobach'e (Berg, 1948; Savvaitova, 1989; Pavlov *et al.*, 1999). In Taimyr and adjacent regions of Siberia, several species with narrow ranges are described. They are represented by Esei charr *S. tolmachoffi* Berg from the Lake Esei (the Khatanga River basin), Taimyr charr *S. taimyricus* Michin from Lake Taimyr (the Nizhnii Taimyr River basin), as well as other charr forms with common or local names or with names given by scientists. Two or more charr forms are distributed in lakes such as Taimyr, Ayan (the Khatanga River basin), Keta (the Pyasina River basin) and Khantaiskoe (the Yenisei River basin) (Pavlov, 1997; Pavlov *et al.*, 1999; Romanov, 2003). Opinions on the origin, range and species status of these forms differ (Berg, 1948; Savvaitova, 1989; Pavlov *et al.*, 1999; Romanov, 2003). According to Romanov (2003), the presence of three charr forms in each of many lakes is connected to three subsequent invasions of different forms in a lake. Data from allozymes (Osinov *et al.*, 1996; Osinov, 2001, 2002) and mtDNA (Brunner *et al.*, 2001; Radchenko, 2003, 2004; Alekseyev *et al.*, 2009; this study) indicate that the representatives of two phylogenetic groups (Atlantic and Siberian) entered into a secondary contact in Taimyr (the Pyasina and Khatanga river basins). It cannot be excluded that one of these phylogenetic groups was represented by populations from different glacial refugia. If two or three forms of charrs developed some mechanism of reproductive isolation during their geographical separation, the consequences of their secondary contact could be different in various areas of Taimyr. Based on available genetic data it is difficult to assess the degree and consequences of hybridization between different forms in Taimyr (Osinov *et al.*, 1996; Alekseyev *et al.*, 2009; see the Lake Lama section in Supporting Information, Data S1). It is possible that morphologically similar forms from different areas, which some authors refer to Boganida charr or Dryagin's charr, may have had a different origin (Savvaitova, 1989). By contrast, phenotypically different forms can have a monophyletic origin.

Based on available allozyme and mtDNA data, the representatives of the Arctic group are absent among different forms of charr from Taimyr. By contrast, the mtDNA data (Brunner *et al.*, 2001; Radchenko, 2003, 2004; this study) reliably indicate that Boganida charr from Lake El'gygytgyn belong to the Arctic group. These findings mean that Boganida charr from Lake El'gygytgyn and Boganida charr from Lake Lama belong to different phylogenetic groups of Arctic charr (Osinov, 2002; Radchenko, 2004). Therefore, their inclusion in a single species *S. boganidae* (Viktorovsky *et al.*, 1981; Chereshnev & Skopets, 1990) is erroneous independently of the opinions connected with the composition of this species.

Using the charrs from Lake El'gygytgyn and from the lakes of Taimyr as an example, we note that a problem of taxonomic status of distinct forms of charrs has not yet been resolved, and a general revision of the genus *Salvelinus* is necessary. The taxonomic problems can be solved only after a more thorough population genetic and phylogenetic analysis of all main forms and populations of charrs over their whole range. Such analysis is especially important for the populations from possible zones of secondary contact between the representatives of different phylogenetic groups or between the populations from different glacial refugia. Unfortunately (according to our estimation), the zones of secondary contact can be found almost in all areas of the occurrence of sympatric forms of Arctic charr.

Salvelinus phylogeny and molecular dating

Based on molecular genetic data, knowledge of the phylogeny of the subfamily Salmoninae has been substantially widened (see reviews: Phillips & Oakley, 1997; Oakley & Phillips, 1999; Crespi & Fulton, 2004), and molecular dating of the main stages of the evolutionary history of salmonids (Salmonidae, Salmoniformes) including the time of whole genome duplication has been achieved (Osinov & Lebedev, 2004). Molecular dating estimates obtained by different authors (Osinov & Lebedev, 2004; Santini *et al.*, 2009; Crete-Lafreniere *et al.*, 2012; Shedko *et al.*, 2012, 2013; Campbell *et al.*, 2013; Macqueen & Johnston, 2014) show a certain correspondence when taking into account their errors and confidence intervals (see Table S1). Priors on times, which include fossil age information, have a strong effect on molecular clock estimates (Inoue, Donaghue & Yang, 2010), and this effect is one of the main causes of differences in the dating obtained by various authors for salmonids. For example, the difference in the dating for the Salmonidae node is connected with the age and calibration priors for fossil *Eosalmo driftwoodensis* and its placement in the phylogenetic tree. In particular, two possible placements in the tree are used for the Middle Eocene *E. driftwoodensis* with the dating between 40 and 45 Mya, connected with uncertain phylogenetic relationships between three subfamilies of the family Salmonidae (Osinov & Lebedev, 2004). The age of deposits of fossils of this species (Wilson & Li, 1999) is estimated as 51–52 Mya (see Moss, Greenwood & Archibald, 2005). Therefore, many authors (excluding Shedko *et al.*, 2012, 2013) have begun to use these values for calibration, and as a result, the estimations of molecular dating in the basal part of the Salmonidae tree have increased (see also Campbell *et al.*, 2013).

A placement of some species in the basal part of the *Salvelinus* phylogenetic tree including *Sl. svetovidovi* has not been resolved. This means that molecular dating obtained for these taxa can be changed to some degree. Divergence within the genus *Salvelinus* began 7.32 Mya (95% BCIs: 5.2, 9.5), similar to previous estimations (see Table S1). The age of the most ancient fossil of *Salvelinus* is more than 10 Mya (Cavender, 1980). The age of the common ancestor for the sister taxa *Sl. svetovidovi* and southern Dolly Varden from Asia is 3.5 Mya (95% BCIs: 1.7, 6.1). To take into account the geological age of Lake El'gygytgyn [approximately 3.6 Mya (Gurov *et al.*, 2007)], the age of the endemic long-finned charr is between 3.6 and 1.7 Mya. Based on our data, the divergence of the main phylogenetic groups of the *Salvelinus alpinus – S. malma* complex occurred in the Middle Pleistocene (1–2 Mya). A replacement of mtDNA of northern Dolly Varden by mtDNA of Arctic charr from Eurasia occurred during this time.

Despite several objections (Emerson, 2007; Bandelt, 2008; Debruyne & Poinar, 2009), some authors believe in the existence of time-dependent rates of molecular evolution when molecular rates observed on intraspecific timescales (< 1–2 Myr) can be an order of magnitude or more greater than the rates on interspesific timescales (> 1–2 Myr) (Ho *et al.*, 2005, 2011). The temporal threshold of a substantial reduction of molecular rates can be different in various animal groups. In particular, it is approximately 200 kyr in galaxiid fishes (Burridge *et al.*, 2008). The analyses with the use of the RLC model (Drummond & Suchard, 2010) do not show significant branch-specific rates and do not allow us to reject the hypothesis of a global clock for *cyt-b* data in *Salvelinus*. Based on RFLP data of mtDNA (Radchenko, 2003), the divergence of ancestral forms of small-mouth charr and Boganida charr began before their invasion into Lake El'gygytgyn. The separation of the ancestor population of Taranets charr from Asia into two geographically isolated populations is probably connected to the events of the last interstadial (64–27 kya, Anderson & Lozhkin, 2011) or glacial period. A large genetic distance (d_A = 0.0029) and related estimation of the divergence time (> 150 kya) between *S. elgyticus* and *S. boganidae* from Lake El'gygytgyn can be attributed to a bottleneck (Nei, 1987; Gaggiotti & Excoffier, 2000) experienced by Boganida charr. An overestimation of the age of these two species can be also associated with a high mutation rate on intraspecific timescales in *Salvelinus* that exceeds the value of the mean rate parameter that is used for the calculation of a divergence time. An increasing rate of molecular evolution is documented in Bayesian analyses for three taxa (*S. levanidovi*, *O. kisutch* and *O. tschawytscha*) using different molecular clock models. Acceleration of molecular evolution based on allozyme data has been

reported before for many *Oncorhynchus* species (Osinov & Lebedev, 2000).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Sample locations (black circles) for *Salvilinus* taxa (according to the data from Table S4). 1, Luomusjarvi Lake; 2, Kanes Laddu Lake; 3, Vyashenskoe Lake; 4, Lama Lake; 5, Leprindokan Lake; 6, Usu Lake; 7, Kamchatka River; 8, Sopochnaya River; 9, Arman' River; 10, El'gygytgyn Lake; 11, Seutakan River; 12, Naivak Lake; 13, Maksi Lake; 14, Juliette Lake; 15, Nauyuk Lake; 16, Mountain Creek; 17, Harrisson Lake; 18, Hill Creek; 19, Longari River; 20, Pochka River; 21, Bol'shoi Somon River; 22, Solov'ovka River and Belaya River; 23, Shikaribetsu Lake; 24, Yama River; 25, Taui River; 26, Twin Lake; 27, Lake William; 28, Walton Lake.

Table S1. Divergence time estimates (mean values ± standard errors or 95% CI in Mya) for some splits in Salmoniformes evolution and whole genome duplication (WGD) in the ancestor for Salmonidae.

Table S2. Indices of genetic variation in ten microsatellite loci of three charr species from Lake El'gygytgyn.

Table S3. Taxonomy, sample location, haplotype abbreviations and frequencies, and GenBank (GB) accession numbers for *Salvelinus* 550-bp *CR* sequences.

Table S4. Taxonomy, sample location, haplotype abbreviations and frequencies, and GenBank (GB) accession numbers for *Salvelinus* 1053-bp *cyt-b* sequences.

Data S1. Lake Lama section.